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Research Article

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Qualitative phytochemical screening and antimicrobial activity of methanol extract of *Maesobotrya barteri* (Baill.) Hutch

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Abstract The stem bark extract of *Maesobotrya barteri* was screened for antimicrobial activity against some pure cultures of bacterial and fungal species. These were carried out by the Plate -hole diffusion method on Mueller – Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungi. The Minimum Inhibitory Concentrations (MICs) of test samples found to be active by the diffusion test were determined based on the macro dilution method. The crude extract showed activity against *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Microsporum spp* and *Candida albicans*. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides. This result confirms its ethnomedicinal use in the treatment of microbial infections.

Keywords Antibacterial, bioactive compounds, *Maesobotrya barteri*, medicinal plants

Introduction

Maesobotrya barteri(bush cherry) is a dioecious tree or shrub belonging to the family Euphorbiaceae with a simple indumentum up to 10 m high. It is a rainforest plant occurring in Sierra Leone, Southern Nigeria, Western Cameroun and Congo Basin [1]. The leaves are alternate, often long-petiolate, stipulate simple, entire or toothed and penninerved. The wood is light and burnt for fuel [2]. *Maesobotrya barteri* bears fruits from April to June, which is up to 1cm long ovoid, and often distinctly pointed [3].

In Nigeria, the plant is known by many local names such as “Oruru” (Benin), “Olowunor Obomodu” (Yoruba), “MiriỌgu” (Igbo) and “Nyanyated” (Ibibio). The fruits are succulent black-purple berries. They are edible and stain the tongue. The seed are often with a conspicuous caruncle with the endosperm present or absent. In Sierra Leone, perhaps due to superstition, the plant is used to make cages in dwellings for twins although the significance is not explained. Stem bark of *Maesobotrya barteri* var. sparsiflora and *Trichilia monadepha* stem bark are boiled together and the decoction is taken for abortion [4]. A bark decoction of *Maesobotrya barteri* is taken in Congo (Brazzaville) for dysentery, urethral discharge and as an aphrodisiac [2]. For a very longtime, *M. barteri* has been in use in the local communities for the treatment of diarrhea, stomachache, dysentery, urethral discharge, venereal disease, jaundice, cough and others [3]. The twigs and small branches are used as chewing stick [5]. Some are eaten as a refreshing delicacy and the fruits serve as a potential source of raw material for juice making that can support the economic and industrial development in Nigeria. Also the fruits enhance frequent waste elimination, including acid, sterols and fat [6]. The fiber is of benefit in diverse diseases [7] and helps lower cholesterol absorption by preventing the formation of plaques [8].

Studies on *Maesobotrya barteri* have shown its major, minor and trace element composition as well as its wide use as chewing sticks in Southern Nigeria [9] and its fruit nutritive value [10]. The use of crushed root and sap of *M. barteri* on the skin externally has been reported to be effective in treating some skin infections [11]. The antimicrobial potential of the crude extract of aerial plant parts of *M. barteri* as well as the corrosion inhibition ability of its leafextract in acid solution have also been reported [12, 13].

To our knowledge, the *in vitro* antimicrobial activity of methanol stem bark extract of *Maesobotrya barteri* has not been reported despite its widespread medicinal use. In the present study, the phytochemical and *in-vitro* antimicrobial activity of stem bark extract of *Maesobotrya barteri* is presented using standard analytical methods.

Material and Methods

Plant materials: The fresh stem barks of *Maesobotrya barteri* (bush cherry) were collected from a bush in Oruk Anam Local Government Area of Akwa Ibom State, Nigeria in the month of June, 2016. The time of collection coincided with the rainy season in Nigeria. Plant identification, authentication and specimen referencing were done at the Department of Botany and Ecological Studies in the Faculty of Science, University of Uyo, Nigeria.

Sample preparation: The stem barks of *M. barteri* were thoroughly washed with distilled water to remove any trace of dirt sticking to the surface of the stem barks. The stem barks were chopped into small pieces and air dried for 7 days. The particle sizes were further reduced by grinding using a Thomas Wiley machine and stored in an air-tight plastic container, properly labeled prior to analyses.

Extraction procedure: The dried pulverized stem barks (500 g) were thoroughly macerated with 99.5 % methanol (5 L) for 7 days at room temperature. The sample mixture was filtered and the filtrate concentrated to dryness in vacuo at 40 ^{o}C using a rotary evaporator to afford a brown colored extract. The extracts were stored in a sealed container and kept in a refrigerator at -4 °C until use. All reagents and chemicals used in this work were of analytical (AnalaR) grade and were sourced from Sigma-Aldrich chemical company, United Kingdom.

Qualitative determination of phytoconstituents: Qualitative tests to identify the constituents of the extract were performed using standard procedures outlined elsewhere [14, 15]. Precisely, screening of alkaloids was carried using Dragendroff's and Mayer's reagents, saponins by Frothing and Fehling's tests. Cardiac glycosides were detected by Liebermann's and Keller-Killiani's tests, tannins by the Ferric chloride test and phlobatannins by hydrochloric acid test. Flavonoids were detected by the magnesium metal/hydrochloric acid test, triterpenes by the chloroform/acetic anhydride/sulfuric acid test and anthraquinones by the benzene/ammonia solution test.

Microorganisms

The bacterial test strains used in this study were *Staphylococcus aureus, Klesbsiella spp, Pseudomonas aeroginosa, Shigella dysenteriae, Salmonella typhi, Esherichia coli, Microsporum spp, Trichophyton spp, Epidermophyton spp, Aspergillus flavus and Candida albicans.* These clinical samples were obtained from St. Luke's Hospital Anua and Microbiology laboratory of University of Uyo, Nigeria. Fungal isolates were collected from the environment and Dermatophyte from some school children all in Uyo Metropolis, Nigeria. The isolates were purified by sub-culturing into their selective medium and thereafter sub-cultured into nutrient agar.

Evaluation of antibacterial and antifungal activities of the extract

Plate-hole diffusion test: The evaluation of antimicrobial activity of the stem bark extract of *Maesobotrya barteri* was carried out by the Plate–hole diffusion method [16] on Mueller-Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungi. Solutions of the extract and fractions were prepared in 10% Tween 80 to concentrations of 100, 50, 25 and 12.5 mg/ml. The innocula of the microorganisms were prepared separately from 12 h broth cultures (Mueller-Hinton broth for bacteria and the Sabouraud dextrose broth for the fungi) and incubated at 37 °C. All culture media and distilled water were sterilized at 121°C for15minutes in an autoclave. These innocula were diluted with sterilized distilled water to obtain a density corresponding approximately to 0.5 of McFarland standard turbidity scale (10^8) colony forming unit "CFU" per mlfor the bacteria and 10^3 spores per ml for fungi) [16]. Each innoculum (0.5 ml) was introduced into the corresponding fluid agar medium homogenized and 25 ml of it poured into sterile plastic petri dishes. The petri dishes were allowed on the flat slab top for the medium to solidify within 30 min. A standard cork borer of 5mm in diameter was used to cut four equidistant uniform wells per plate on the surface of different plates into which was added 50µl solution of the extract at varying concentrations 12.5, 25, 50 and100 mg/ml.

The reference drugs were Gentamicin, batch 20070402 (0.4mg/ml) and Nystatin batch 04D05 (500µg/ml). The plates were incubated at 37 °C for 24 and 48 h for the bacteria and fungi respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition around the hole. Each test concentration had three replications. The results were recorded as the mean diameter of the zones of growth inhibition surrounding the discs [17]

Determination of minimum inhibitory concentrations (MIC) using macrodilution method

The Minimum Inhibitory Concentrations (MICs) of test samples found to be active by the diffusion test were determined based on the macrodilution method [16] with some modifications as follows. The test extract/fractions were dissolved in 10% Tween 80 to give a stock concentration of 100 mg/ml and serially diluted (two-fold) in a series of test tubes to a working concentration ranging from 1.560 to 100 mg/ml using nutrient broth supplemented with 10% glucose and 0.05% phenol red (colour indicator). These were later inoculated with 0.2ml suspension of the test organisms. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

Table 1: Phytochemical composition of methanol stem bark extract of *Maesobotrya barteri*

Phytoconstituents	Detection
Alkaloids	Present
Tannins	Present
Terpenes	Present
Cardiac glycosides	Present
Flavonoids	Present
Saponins	Present
Anthraquinones	Absent
Phlobatannins	Absent
Deoxy sugar	Absent

Table 2: Inhibition zone diameter, IZD (mm) of methanol extracts of *Maesobotrya barteri* on some test organisms

Test Organisms	Zones of inhibition (mm) with different concentrations of methanol extract				Gentamycin 0.4 mg/ml	Nystatin500 µg/ml
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml		
	ME	ME	ME	ME		
S. aureus	18	14	12	10	23	-
Klebsiellaspp	-	-	-	-	16	-
P. aeroginosa	-	-	-	-	12	-
S. dysenteriae	15	12	10	8	7	-
S. typhi	10	8	6	6	7	-
E. coli	-	-	-	-	19	-
Microsporumspp	10	-	-	-	-	15
Trichophytonspp	-	-	-	-	-	22
Epidermophytonspp	-	-	-	-	-	29
A.flavus	-	-	-	-	-	20
C. albicans	20	17	14	12	-	24

Table 3: Result of minimum inhibitory concentration (MIC) of stemb ark extract of *M. barteri*

Test Organisms	Extract Concentration (mg/ml) Methanol
S. aureus	6.25
S. dysenteriae	6.25
S. typhi	7.25
Microsporum spp	55
C. albicans	3.25

Results

Phytochemical screening

The result of the phytochemical screening of the methanol extract of stem bark of *M. barteri* is shown in Table 1. The result shows the presence of alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides by qualitative methods. Anthraquinones, phlobatannins and deoxy sugar were found to be absent.

Antimicrobial activity

Table 2 shows the diameters of the zones of inhibition exhibited by the crude extracts at various concentrations employed. The zones of inhibition of methanol extract of *M. barteri* showed remarkable activities against five of the eleven organisms tested. The zones of inhibition were compared with these standard drugs; gentamycin and Nystatin. The crude extract showed activity against *Staph. aureus,* a gram-positive bacteria, *S. dysenteriae* and *S.typhi* which are gram-negative bacteria. The crude extract was active against only two of the five fungal species tested namely; *Microsporum spp* and *C. albicans.*

The results of minimum inhibitory concentrations (MIC) of the crude extract are shown in Table 3. The lowest MIC (3.25 mg/ml) for the methanol extract of *M. barteri* was recorded against *Candida albicans* while the highest MIC (55 mg/ml) was recorded against *Microsporum spp*.

Discussion

The phytochemical screening of the stem bark extract shows the presence of alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides by qualitative methods. Anthraquinones, phlobatannins and deoxy sugar were found to be absent in the crude extract.

The medicinal properties of these secondary metabolites are quite numerous and have been well documented elsewhere [18-22]. The presence of these bioactive compounds in the leaves of *M. barteri* corroborates the various pharmacological activities of this plant and supports its widespread use in traditional medicine.

Three of the bacterial strains tested (Gram-positive and Gram-negative were sensitive to the methanol stem barke xtract of *Maesobotrya barteri*. This result is in agreement with an earlier report [12] on the activity of the triterpene β-Amyrin from the aerial parts of *Maesobotrya barteri* against *Staphylococcus aureus*, *Shigella dysenteriae* and *Salmonella typhi*. In contrast to this report, the stembark extract of *M. barteri* did not show any activity against *Klebsiellaspp, Escherichia coli* and *Trichophyton spp.* In a similar comparison for anti-fungal activity, the result of this study agrees in part with an earlier report [12] which showed activity of the triterpene β-Amyrin from the aerial parts of *Maesobotrya barteri* against *Microsporum spp* but disagrees because the crude methanol extract of the stembark of *M. barteri* also shows activity against the fungus *Candida albicans.* The extract has fungistatic activity on only *Candida albicans* of all the fungi tested probably due to the morphological differences existing between the yeast-like *Candida* and the other filamentous fungi. The MICs obtained varied from 3.25 to 55 mg/ml for the crude extract of the stem bark of *M. barteri.* The MIC values and the antimicrobial spectrum of crude extract of the stem bark of *M. barteri* indicate a remarkable antimicrobial potency which can be exploited as promising antimicrobial agent. It is observed that the commercially perfected antibiotics have larger zones of inhibition, indicating that drugs should be used in high purity and commercially perfected forms. Similar trends were observed for the antimicrobial activity of methanol extract leaf extract of guava [23].

Conclusion

In conclusion, the results of this study show that the stem bark extracts of *Maesobotrya barteri* possess significant antimicrobial activity which justifies its use in traditional medicine in the treatment of microbial infection. The bioactive compounds present in this extract may in part be responsible for the antimicrobial activity observed in this study. Therefore, it will be interesting if the bioactive compounds are isolated and characterized for future beneficial use.

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